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MINIREVIEW

Detection of Brucellae in Blood Cultures

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Brucellae are small gram-negative nonmotile coccobacilli which can be isolated as part of the normal flora of the genitourinary tract of a variety of wild and domestic animals including cows, goats, sheep, pigs, and dogs (22, 34). The organism is strictly aerobic, nonencapsulated, and catalase and oxidase positive; it does not ferment carbohydrates and has variable urease activity (34). Based on DNA homology, it has been demonstrated that all six members of the genus are, in fact, serovars of a single species of which four, namely, *Brucella abortus*, *B. suis*, *B. canis*, and especially *B. melitensis* are able to cause human infections (34). Brucellosis is usually transmitted to humans by direct contact with infected animals or by ingestion of unpasteurized dairy products (34). In addition, occupational exposure of abattoir workers, veterinarians, and laboratory technicians may result in transmission of the disease through contaminated aerosols.

Brucellae are capable of evading host defense mechanisms, surviving as intracellular organisms, and are able to cause prolonged morbidity, relapses, and long-term sequelae. Brucellosis is a systemic infection that may affect any organ system in the body (28, 29, 34). Because of the wide spectrum of its clinical manifestations, brucellosis may mimic other infectious and noninfectious conditions and, therefore, the diagnosis of the disease is frequently delayed or even missed (29, 34).

Brucellosis continues to affect large human populations living in rural areas in Mediterranean, Middle East, and Latin American countries where the organisms are endemic (2, 13, 14, 27–29, 31–34). In developed countries, the incidence of human brucellosis has declined in the last 50 years as the result of infection control measures, and in these countries most cases represent occupational disease, travel-acquired infections, or accidental laboratory exposure (34). Because of the low prevalence of brucellosis in the developed world, microbiology laboratories in these regions are frequently unfamiliar with the diagnostic tools available for the isolation of the organism. The purpose of this review is to summarize published information on the performance of blood culture techniques for the detection of *Brucella* organisms. Because anaerobic conditions do not adequately support the growth of brucellae, only data on the performance of aerobic media will be included.

ROLE OF BLOOD CULTURES IN DIAGNOSIS OF HUMAN BRUCELLOSIS

Although a presumptive diagnosis of brucellosis can be made by demonstrating high or rising antibody titers to *Brucella* antigens, isolation of the organism from blood, bone marrow, or tissue cultures is the only irrefutable proof of the disease (29, 34).

Overall, blood cultures are positive in 53.4 to 90% of patients with brucellosis but the chances of successful isolation of the organism decrease over time (14, 25).

Because of the suboptimal recovery rate of brucellae from blood, it has been suggested that cultures of bone marrow (1, 11, 14, 25, 34), liver tissue (6, 9), or lymph nodes (21) may improve the recovery rate of the organism. The rationale for these alternative approaches is that *Brucella* organisms survive the intracellular killing by phagocytes and polymorphonuclear leukocytes and localize in the reticuloendothelial system (14, 29, 34).

The relative merits of culture of specimens other than blood, however, remain unclear. Ganado and Bannister demonstrated that in 20% of patients with brucellosis with positive bone marrow cultures the organism could not been isolated from blood (11). Gotuzzo et al. reported that, among 50 patients with proven brucellosis detected by cultures of blood, bone marrow, or both, bone marrow cultures were positive in 46 of 50 (92%) patients, whereas blood cultures were positive in only 35 (70%) (14). Despite the small volume of bone marrow cultured (<1 ml) compared to the much larger blood volumes (5 to 10 ml), brucellae grew more rapidly from bone marrow cultures, suggesting that higher bacterial loads may be present in this type of specimen. Magill and Killough found that blood cultures were more reliable (sensitivity of 90%) than bone marrow cultures (sensitivity of 40%) (16), and Shehabi et al. found that, in their experience, blood cultures had a sensitivity of 44.4% compared to 27.7% for bone marrow cultures (28).

Because brucellae are intracellular organisms and the serum of patients with brucellosis may have antibacterial activity, culture of the blood clot, where organisms circulating into leukocytes may be trapped, has been attempted. However, Escamilla et al. found these cultures to be less sensitive and more labor-intensive when compared with a conventional blood culture method (7).

BROTH CULTURE METHODS

Manual monophasic blood culture methods. Although the isolation of brucellae from normally sterile sites may be achieved by using routine culture techniques, detection of the organism in clinical specimens is frequently hampered by its slow growth. Based on the experience gained with traditional methods, incubation of blood cultures for 30 days instead of the routine 1-week period and performance of blind subcultures have been advocated to maximize the recovery of these fastidious organisms because brucellae may be present in blood culture broths without visible evidence (1, 18). The limitations of this approach are obvious: performance of repeat

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TABLE 1. Time-to-detection of *Brucella* spp. by nonautomated blood culture systems

System	Incubation for ≥14 days	Blind subculture	Patients (*n*)	Positive cultures (*n*)	Detection by system within:				Reference
					4 days		7 days		
					n	%	*n*	%	
Castañeda	No	Yes	?	49	?[a]	?[a]	?[a]	?[a]	27
Hemoline	Yes	Yes	18	28	?[b]	?[b]	?[b]	?[b]	12
	Yes	Yes	19	19	5	26.3	14	73.7	26
In-house lysis-centrifugation	NA[c]	NA	?	14	14	100.0	14	100.0	8
	Yes	NA	15	15	15	100.0	15	100.0	15
Isolator lysis-centrifugation	NA	NA	7	7	6	85.7	7	100.0	19
	No	NA	11	22	19	86.4	22	100.0	33

[a] Twelve (24.5%) were detected by days 5, and forty-nine (100%) were detected by day 10.
[b] Mean time-to-detection, 7 days.
[c] All positive cultures were detected within 7 days. NA, not applicable.

subcultures is labor-intensive, keeping bottles for several weeks requires a large incubation space, and confirmation of the disease is delayed. In addition, unless physicians and laboratory personnel are aware of the possibility of brucellosis, blood cultures are routinely discarded after a 5- to 7-day incubation period and, therefore, isolation of the slow-growing brucellae may be missed.

Biphasic methods. To avoid the need to make repeat subcultures, a biphasic medium consisting of a solid and a liquid phase in the same blood culture bottle was developed by Castañeda and others (1, 18, 24, 25). After inoculation, the air in the bottle is replaced by a mixture of air with added 10% CO_2 and tilted so that the liquid flows over the solid medium, and then the bottle is incubated in the upright position and examined every 3 days (1, 24, 25). Any colonies that appear in the solid media should be subcultured and identified. If no colonies are observed, bottles are tilted again and reincubated, repeating the 3-day cycle for at least 35 days (1, 24, 25).

In a study by Gotuzzo et al. (14), cultures processed by the Castañeda method usually became positive within 1 week (mean time-to-detection of 4.3 and 6.7 days for bone marrow and blood specimens, respectively), and no new positive bottles were detected after 15 days of incubation. In another study, however, the time to detection was more prolonged: the majority of the blood culture bottles required 7 to 21 days of incubation, and 2% of bottles were detected as positive after day 27 (22). Differences in the clinical features of the patients' population or in the quality of the media used may account for the discrepancies found in the results of these two studies.

Serrano et al. (27) obtained 83 blood culture sets from 42 patients with positive *Brucella* agglutinin titers. Five milliliters of blood was inoculated into a Castañeda flask, and an identical volume was inoculated into an aerobic BACTEC 460 bottle (Johnston Laboratories, Towson, Md.). Both media were incubated for 10 days and subjected to blind subcultures on chocolate-agar plates on days 5 and 10. On day 5, 14 cultures were positive. The biphasic medium detected 12 positive cultures (85.7%), and the BACTEC bottle detected 10 positive cultures (71.4%), of which only 2 were detected radiometrically and the remaining by subculture only. After 10 days of incubation, 49 bottles were positive with the biphasic medium, whereas the radiometric medium detected 56 ($P > 0.05$), of which only 27 were detected by the instrument. Unfortunately, no data on the performance of the Castañeda flask without the subculture step were reported.

In recent years, an enriched biphasic flask (Hemoline performance diphasic medium; bioMerieux, Marcy l'Etoile, France) has been developed for the routine diagnosis of bacteremia. In a study by Garcia-Rodriguez et al., the performance of the Hemoline system was compared to that of the BACTEC NR (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.) (12). Although the Hemoline medium was superior to the automated system in terms of sensitivity and time-to-detection of brucellae, an average incubation of 7 days was required to isolate the organism from the biphasic flask (12).

In a prospective study, Ruiz et al. evaluated the performance of the Hemoline system for the recovery of *B. melitensis* (26). Flasks were inoculated with 10 ml of blood obtained from patients with suspected brucellosis and incubated for 21 days. A single blind subculture on agar plates of all negative media was performed at the end of the incubation period. The median time-to-detection of 19 positive blood cultures was 5 days. However, 4 of 19 (21.1%) cultures were detected after the seventh day of incubation (26). Performance of the Hemoline and other nonautomated blood culture methods for the recovery of *Brucella* spp. is summarized in Table 1.

Lysis centrifugation: in-house methods and Isolator blood culture system. Braun and Kelsh were the first to report use of a membrane filter technique for recovery of *Brucella* spp. from blood of rabbits experimentally inoculated with the organism (4). A heparinized blood specimen was subjected to osmotic lysis and filtered through a sterile Millipore filter under negative pressure. Filters were then placed on the surface of a solid medium, and bacteria retained in the filter grew as colonies on the agar surface. Despite promising results, the method never gained popularity because it was cumbersome and labor intensive, and filters plugged with formed elements of the blood.

This original approach was modified by osmotic lysis of blood cells followed by concentration of organisms by a centrifugation step and dispersion of the concentrate on the surface of agar media (8, 15). In 1984, Etemadi et al. evaluated this lysis centrifugation procedure and compared it with the Castañeda medium for the recovery of *B. melitensis* from blood and other normally sterile body fluids (8). Fourteen blood cultures, two bone marrow cultures, and two cerebrospinal fluid cultures were found to be positive by the lysis-centrifugation method within 48 h, whereas all eighteen cultures were found to be negative by the Castañeda procedure after 21 days of incubation (8).

In 1991 Kolman et al. obtained a single blood culture from 54 patients with serologically confirmed brucellosis (15). A portion of the blood sample was inoculated into an the aerobic BACTEC 460 bottle, and the remaining volume was subjected to an in-house lysis-centrifugation procedure (15). The automated blood culture system was superior in terms of sensitivity

TABLE 2. Time-to-detection of *Brucella* spp. by automated blood culture systems

System	Incubation for ≥14 days	Blind subculture	Patients (*n*)	Positive cultures (*n*)	Detection by instrument within:				Reference
					4 days		7 days		
					n	%	*n*	%	
BACTEC 460	Yes	Yes	15	15	0	0.0	15	100.0	2
	No	Yes	1	3	0	0.0	0	0.0	17
	No	Yes	?	56	?[a]	?[a]	?[a]	?[a]	27
BACTEC NR	Yes	Yes	8	12	0	0.0	1	8.5	12
	Yes	No	6	12	0	0.0	0	0.0	19
	Yes	No	19	19	0	0.0	?[b]	?[b]	15
	No	No	?	58	44	75.9	58	100.0	13
	Yes	Yes	21	27	2	7.4	21	77.7	31
	No	No	27	42	6	14.3	42	100.0	31
	NA[c]	Yes	1	2	0	0.0	?	?	35
	Yes	Yes	1	9	0	0.0?	?[d]	?[d]	35
BACTEC 9000	No	No	?	30	30	100.0	30	100.0	13
	Yes	Yes	16	42	37	88.1	41	97.6	32
	Yes	No	97	97	69	71.1	94	96.9	3
	Yes	Yes	18	18	10	55.6	18	100.0	26
Vital	Yes	Yes	18	18	1	5.6	8	44.4	26
BacT/Alert	Yes	Yes	5	11	1	9.1	1	9.1	5
	NA[d]	NA[d]	1	3	3	100.0	3	100.0	30
	Yes	Yes	5	9	9	100.0	9	100.0	23

[a] Two cultures (3.6%) were detected by day 5, and twenty-seven (48.2%) were detected by day 10.
[b] Mean time-to-detection, 14 days (range, 7 to 30 days).
[c] All positive cultures were detected within 7 days. NA, not applicable.
[d] All positive cultures were detected within 20 days.

and recovered *B. melitensis* in 19 of 54 (35.2%) cultures, whereas the lysis-centrifugation method detected only 15 (27.8%). Time-to-detection, however, was significantly shorter for the lysis-centrifugation method and detected brucellae after an average of 3.5 days (range, 2 to 4 days) versus an average of 14 days (range, 7 to 30 days) for the BACTEC 460 system.

In 1993, Navas et al. reported that the commercial Isolator Microbial Tube (Wampole Laboratories, Cranbury, N.J.) blood culture system reduced the time-to-detection of brucellae to 2 to 5 days (19). In a prospective study, 10 ml of blood obtained from patients with suspected brucellosis was inoculated into an Isolator Microbial Tube, and two 5-ml aliquots were inoculated into one aerobic (NR6A) and one anaerobic (NR7A) BACTEC NR660 blood culture system bottle. The lysis technique detected all seven positive cultures, whereas the broth method missed one positive blood culture set. The time-to-detection by the BACTEC NR660 was also more prolonged, ranging from 17 to 29 days, with a mean of 20.6 days. It should be pointed out, however, that although an equal blood volume was used to inoculate each system, because anaerobic bottles cannot sustain the growth of brucellae, for practical purposes the effective blood volume inoculated into the BACTEC system was only one-half of that seeded in the Isolator plates.

In our own experience, 15 of 22 (68.2%) blood cultures processed by the Isolator system were detected positive for *B. melitensis* after 72 h of incubation (33). When compared with the automated BACTEC 9240 system, the Isolator Microbial Tube was, however, inferior in terms of sensitivity and time-to-detection (see Automated Blood Culture Systems section).

Automated blood culture systems. Over the last few years, experience on the isolation of *Brucella* spp. by use of automated blood culture systems has been accumulating at a slow pace. Although the disease is still prevalent in developing countries, the use of modern bacteriologic techniques in these areas is limited, whereas in the developed world, where use of automated blood culture systems is widespread, brucellosis has been successfully eradicated. Medical literature is frequently limited to retrospective reports of single cases or small outbreaks of disease among travelers to areas where the organism is endemic, and prolonged incubation of bottles and blind subcultures of negative media were not always done. Published reports on the performance of automated blood culture methods are summarized in Table 2.

Factors influencing detection of brucellae by automated systems. It has been traditionally assumed that the concentration of circulating brucellae in the blood of infected patients is low, although solid data on this subject are scarce (10). In our own experience, the magnitude of *Brucella* bacteremia as determined by the Isolator Microbial Tube system in children with acute infection is extremely variable, ranging from 1.3 to >1,000 CFU/ml, with a median of 88 CFU/ml (34). Time-to-detection correlated inversely with the concentration of viable organisms in the blood sample, validating the results of experimental studies (30, 35).

Brucella organisms have a comparatively long (2.5 to 3.5 h) doubling time compared to other human pathogens (10). However, the key explanation for the delayed detection of the organism by some automated blood culture systems appears to be the slow release of CO_2 by members of the genus. In a series of in vitro studies with the BacT/Alert system (Organon Teknika Corporation, Durham, N.C.), a slow production of CO_2 by *B. melitensis* compared to *Escherichia coli* and *Staphylococcus aureus* was demonstrated, and the peak values obtained were of lower magnitude (30). In a study by Gamazo et al., broth culture media showed visible turbidity, indicating a

large bacterial concentration, on average 24 h earlier than detection of positivity by the BACTEC 730 instrument (10).

The effect of adding different CO_2 sources (pyruvate, alanine, glutarate, urea, glucose, and erythritol), as well as changing the pH, on the automated detection of *B. melitensis* was also studied (10). Only the addition of alanine and pyruvate resulted in a mild increase in the production of CO_2 by growing organisms. Lowering of the pH of the medium from 7.2 to 6.2, in addition to supplementation with pyruvate, resulted in a more marked increase. Although these experiments suggest that modifications in the blood culture medium may shorten the time-to-detection of *Brucella* spp. from blood, this approach cannot be recommended because changes in the broth formulations may not necessarily support growth of other bloodborne pathogens.

In the same study, the detrimental effect of the anticoagulant sodium polyethol sulfonate (SPS) contained in the medium was observed. However, there are no good alternatives to the antiphagocytic, anticomplementary, and aminoglycoside-neutralizing effects of this compound. In the 9000 series of BACTEC blood culture system media, the concentration of SPS has been reduced to 0.025% compared to 0.035% in the NR660 and BacT/Alert media, a fact that may partially explain the better performance of the former system for detecting brucellae (3).

Radiometric detection of brucellae. The BACTEC 460 was the first in a series of automated blood culture systems developed in the last two decades. Published experience on the use of this system for the recovery of brucellae from blood is limited (2, 15, 17, 27). In 1984, Arnow et al. investigated an outbreak of *B. melitensis* infections among travelers to Spain (2). Overall, 15 of 19 (78.9%) blood cultures obtained from six different patients were positive, and all grew the organism between the fourth and eighth days of incubation. Three years later, brucellosis was diagnosed in a traveler to Iraq (17). *B. melitensis* was isolated from a blind subculture performed in a 3-day-old radiometrically negative blood culture bottle. Despite incubation and monitoring of the bottle for a total of 9 days, CO_2 production never reached the threshold value.

Infrared detection system. Published experience with the use of infrared detection technology (the BACTEC NR instruments) for the detection of *Brucella* spp. is also limited (12, 13, 15, 19, 31, 35). Zimmerman et al. recovered *B. abortus* by subculture of two 5-day-old blood cultures and from a 7-day-old bone marrow culture inoculated into aerobic BACTEC NR bottles (35). Once the diagnosis was made, 15 additional bottles, including aerobic, osmotically stabilized (hypertonic), and anaerobic media were inoculated and processed by the automated instrument. All five aerobic bottles became positive between days 7 and 20 and four of five hypertonic media were found to be positive within 20 days, whereas all five anaerobic bottles remained negative.

In a Spanish study, the BACTEC NR blood culture system was clearly inferior to the Hemoline biphasic medium (12). BACTEC NR bottles and biphasic flasks were monitored for 21 days, and negative media were blindly subcultured at the end of the period. The Hemoline system detected 28 positive cultures from 18 patients after an average 7-day incubation. The BACTEC NR system detected only 12 positive bottles and missed 10 patients. Moreover, 11 of these 12 bottles gave negative infrared readings during the 3-week monitoring period, and the organism was detected by subculture only (12).

In the study by Navas et al., only 12 of 16 (75%) blood culture sets drawn from seven patients with brucellosis were positive and missed the diagnosis in one patient, whereas the comparator blood culture system (Isolator Microbial Tube) gave an accurate diagnosis in all seven patients (19). The average time-to-detection of brucellae by the BACTEC NR system was 3 weeks (range, 17 to 29 days) (19). Using the same blood culture system, Gedikoglu et al. reported isolation of brucellae in 22 patients with a median detection time of 72 h (13). In this study, no blind subcultures of negative bottles were performed and no bottle was incubated for more than a week, so the results do not allow assessment of the sensitivity of the system for detecting brucellae within the routine blood culture schedule.

To evaluate the performance of the BACTEC NR blood culture system for the detection of *B. melitensis* within the routine 1-week blood culture protocol, we conducted a prospective 24-month study in an area of southern Israel where the organism is endemic (31). Blood cultures obtained from patients with suspected brucellosis were monitored by the blood culture instrument and blindly subcultured once per week for 4 weeks, and the fraction of blood cultures positive for *B. melitensis* detected by the instrument within the first week was determined. During the study period, 27 of 373 (7.2%) blood cultures, obtained from 21 patients, were positive for the organism. Twenty-one (78.8%) of these positive cultures were detected by the BACTEC NR instrument within 7 days, and six positive cultures (22.2%) were detected by subculture after 2 or 3 weeks of incubation, confirming that prolonged incubation and periodic performance of subcultures of negative bottles were still needed to maximize the recovery of *B. melitensis* by the BACTEC NR blood culture system.

It is noteworthy that in the same study, *B. melitensis* was incidentally isolated within the routine 7-day protocol from additional 42 blood cultures, drawn from 27 patients in whom the diagnosis of brucellosis was not suspected (31). This observation reinforces the need to detect brucellae within the routine blood culture protocols instituted by most clinical microbiology laboratories.

Continuous monitoring systems. The experience with the use of the BacT/Alert blood culture system for the recovery of brucellae is rather limited (5, 23, 30). In 1992, Solomon and Jackson detected *B. melitensis* in the blood of a traveler to the Middle East after an incubation period of only 2.8 days (30). In another study, Casas et al. obtained blood cultures from six patients with confirmed brucellosis (5). Bottles were monitored by the BacT/Alert instrument for 10 consecutive days and were then transferred to a regular incubator for 10 additional days. Blind subcultures were performed on days 10 and 20. Only one of nine positive bottles was detected positive by the automated instrument after 2.9 days of incubation. Seven other bottles were detected positive by subculture on day 10, and the remaining one was detected on day 20 (5). Although the results of this study suggested that the BacT/Alert blood culture system may be able to rapidly detect brucellae, Roiz et al. reported that in their experience all 9 cultures obtained from five patients yielded the organism within 88.4 h (23). In addition, a blood culture bottle inoculated with pancreatic fluid of one of the patients was detected positive after only 13.3 h (23).

In 1996, Gedikoglu et al. summarized the results accumulated in a Turkish hospital with the BACTEC 9120 system with a 7-day protocol (13). Thirty blood cultures, obtained from 15 different patients, grew *B. melitensis*. All positive cultures were detected within 84 h of incubation. Using the BACTEC 9240 larger version of the system and a similar protocol, we detected 59 of 77 (76.6%) consecutive cultures positive for brucellae within 4 days of incubation (unpublished data).

Despite these impressive results, limiting incubation of blood culture bottles drawn from patients with suspected bru-

cellosis to the routine 1-week period instituted in most laboratories cannot be routinely recommended unless it is firmly demonstrated that by adoption of this approach no significant number of positive cultures are missed.

This issue was specifically addressed in a prospective study recently conducted in febrile children in southern Israel (32). According to the traditional recommendation, inoculated Peds Plus/F (aerobic pediatric) blood culture bottles were monitored by the BACTEC 9240 instrument for 4 consecutive weeks, and blind subcultures of negative bottles were performed once a week (32). Of total of 2,579 blood cultures drawn, 42 (1.6%) were positive for *B. melitensis*. Of the 42, 41 (97.6%) positive cultures were detected by the BACTEC 9240 instrument within 2 to 6 days. A single positive culture was missed by the instrument and detected by blind subculture performed on day 7. Cumulative percentage rates were 23.6, 78.9, 86.8, 92.1, and 97.4% for days 2, 3, 4, 5, and 6, respectively.

Similar results were obtained in a study conducted in Saudi Arabia among a population of children and adult patients (3). Standard BACTEC 9240 aerobic/F (for culturing blood of adults) and Peds Plus bottles (used for pediatric patients) were incubated for up to 21 days. No blind subcultures of negative bottles were performed. Overall, 90 of 97 (92.7%) positive cultures (including 85 *B. melitensis* and 12 *B. abortus* isolates) were detected by the BACTEC instrument within 5 days of incubation. Only three (3.1%) positive bottles were detected after the seventh day (two on day 8 and one on day 9).

The performance of three blood culture systems (Hemoline performance diphasic medium, BACTEC 9120, and Vital Aer [bioMerieux]) for the recovery of brucellae was compared in a prospective study involving 19 positive blood cultures obtained from Spanish patients (26). Overall, the Hemoline medium detected all 19 positive cultures (sensitivity, 100%), whereas the BACTEC 9120 and the Vital systems missed one positive culture each (sensitivity, 94.7%). By using a 5-day incubation protocol, 47.4, 78.9, and 10.5% cultures were detected by the three blood culture systems, respectively. When the protocol was extended to 7 days, the results were 73.7, 94.7, and 47.4%, respectively, indicating that the BACTEC system was significantly faster than the comparators ($P < 0.05$).

The sensitivity and time-to-detection of *B. melitensis* by the BACTEC 9240 and the Isolator blood culture systems were also compared in a prospective study. Equal blood volume samples, obtained from children with suspected brucellosis, were inoculated into a BACTEC 9240 Peds Plus aerobic bottle and into an Isolator 1.5 Microbial Tube (33). Overall, 122 pairs of blood cultures were obtained, and 28 (23%) were found to be positive by at least one method. The BACTEC 9240 system detected all 28 positive cultures (sensitivity, 100%), and the Isolator system detected 22 positive cultures (sensitivity, 78.6%) ($P < 0.023$). Among those 22 cultures positive by both methods, 21 (95.5%) and 15 (68.2%) were found to be positive within 3 days by the BACTEC and the Isolator systems, respectively; 8 (36.4%) were detected at least 1 day earlier by the BACTEC instrument, and the remaining 14 were detected by the two systems on the same day ($P < 0.045$). It was concluded that the BACTEC 9240 blood culture system was more sensitive than the Isolator Microbial Tube for the detection of *B. melitensis* and was superior in terms of time-to-detection of the organism.

CONCLUSIONS

In the past, the diagnosis of brucellosis was hampered by the slow growth of the organism and the lack of a suitable commercial blood culture system. To maximize recovery of this fastidious bacterium from blood, use of a biphasic medium, prolonged incubation, and periodic performance of blind subcultures were traditionally recommended. Development of automated blood culture systems and technical improvements have resulted in gradual increase in the sensitivity of methods and shortening of detection time of *Brucella* spp. Nowadays, use of aerobic bottles of automated blood culture systems and especially of the BACTEC 9000 instruments makes possible the diagnosis of more than 95% of positive cultures within the routine 7-day blood culture protocol, and performance of subcultures of negative media is no longer necessary. This method is more sensitive than the Isolator Microbial Tube and permits earlier detection of the organism.

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